

U.S.S.N 09/910,120

Ault-Riche *et al.*

PRELIMINARY AMENDMENT ATTACHMENT

MARKED-UP COPY OF FIGURES

Sorting by pools

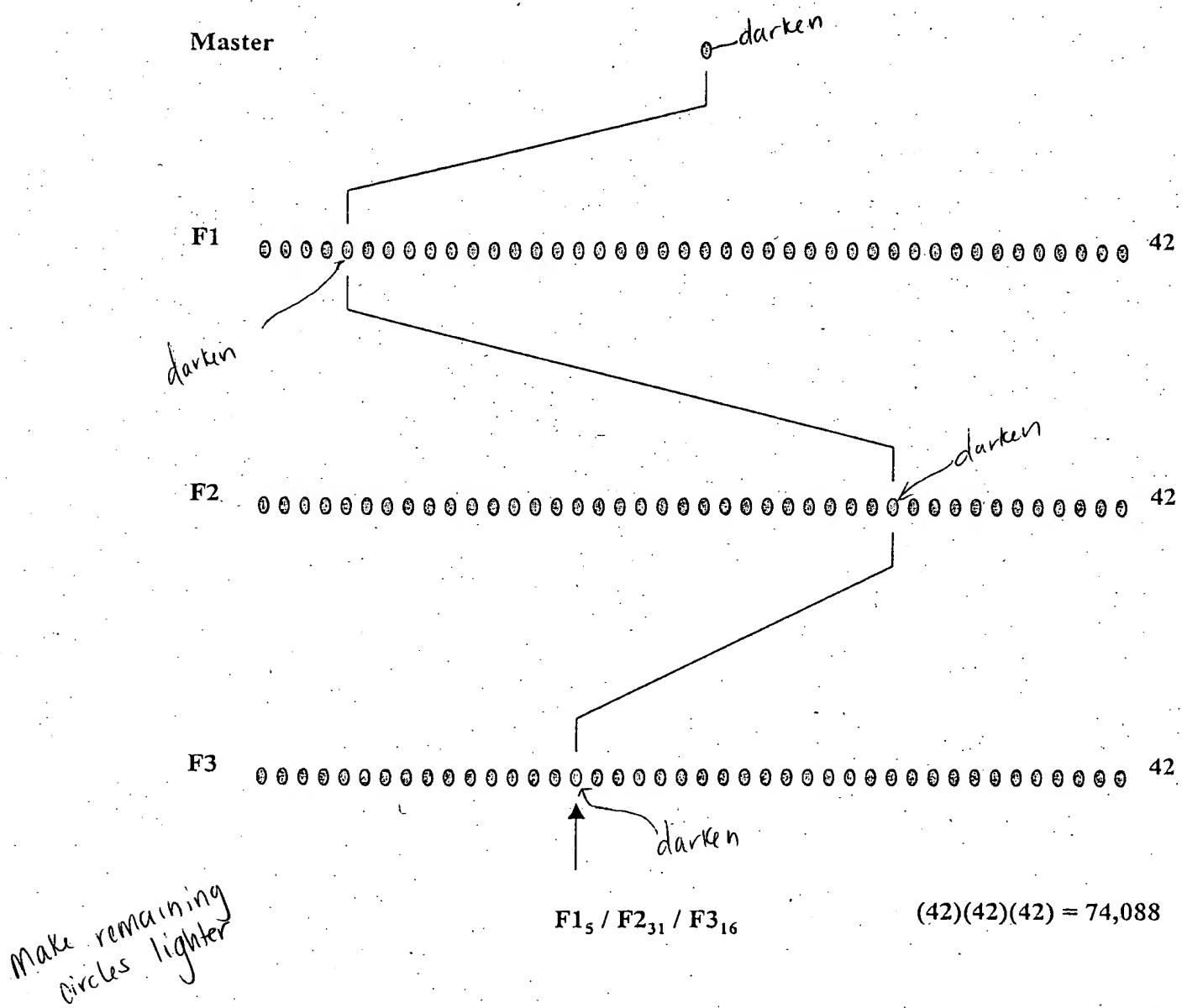


FIGURE 1

HELLER EHRMAN WHITE & McAULIFFE LLP
Sheet 2 of 23
Applicant: Ault-Riche et al.
DKT. No. 25885-1751
Priority claimed to 60/219,183
For: COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Sorting by pools: Decreasing pool diversities

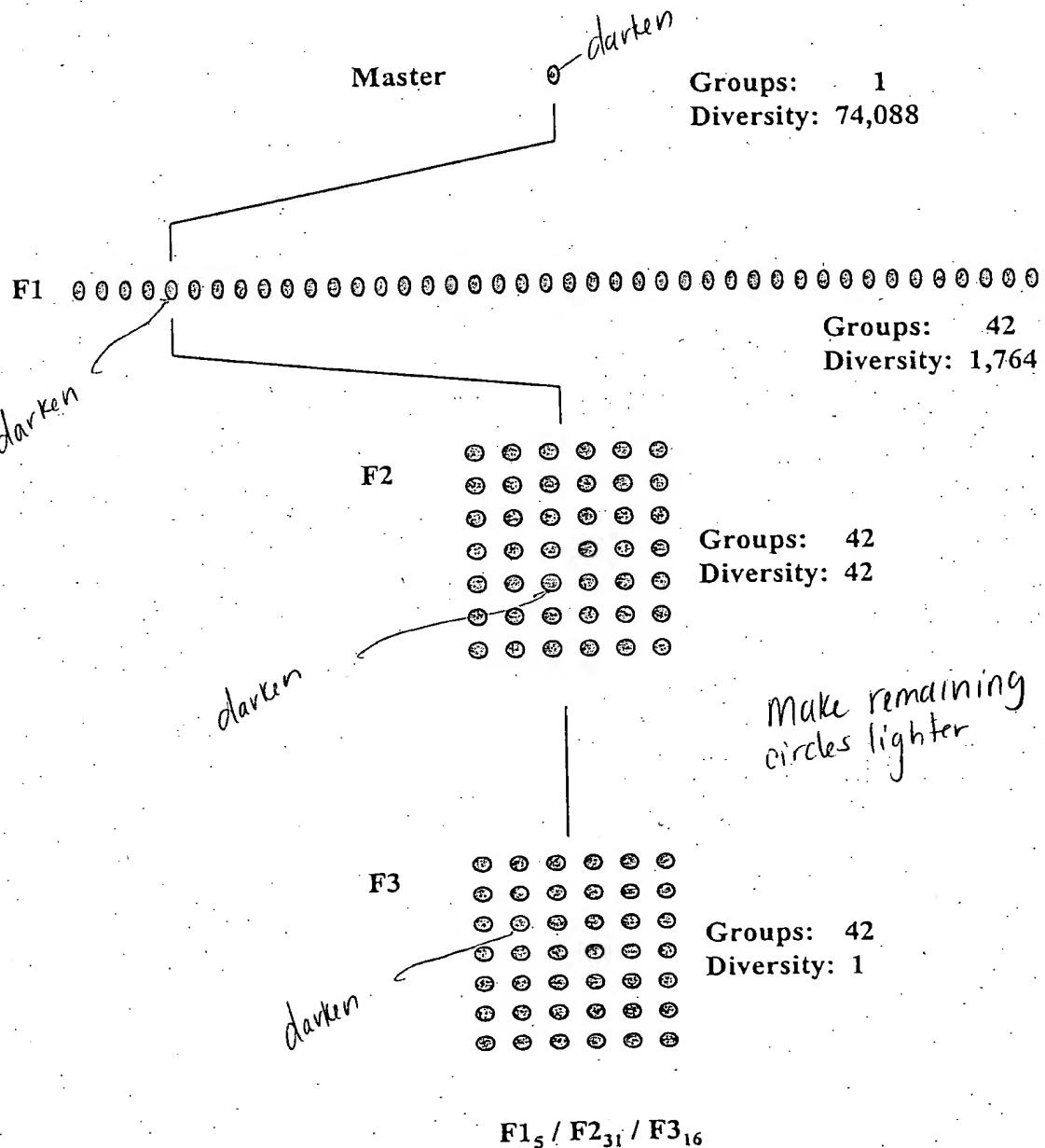


FIGURE 2

Applicant: Ault-Riche et al.
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Sorting by pools: Screening large diversity libraries

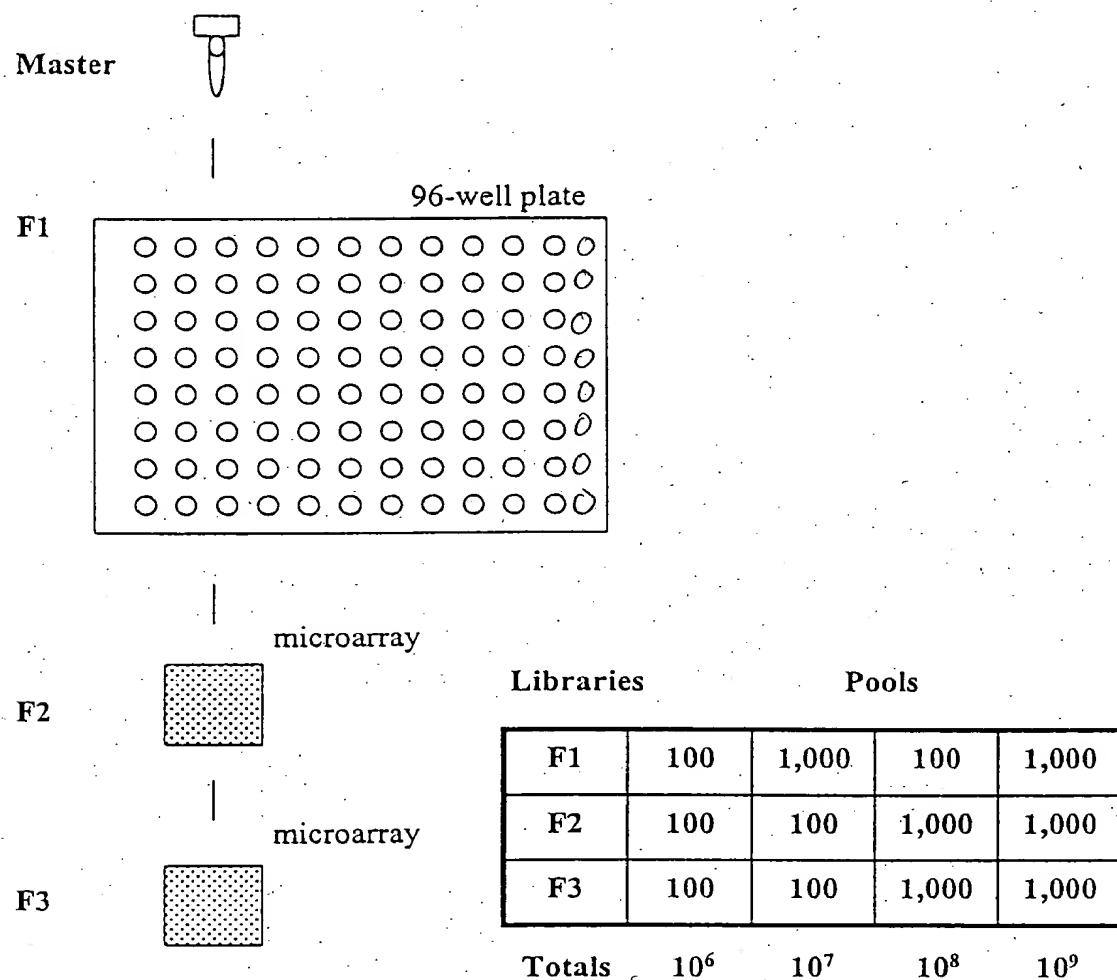


FIGURE 3

Searching a mutation library

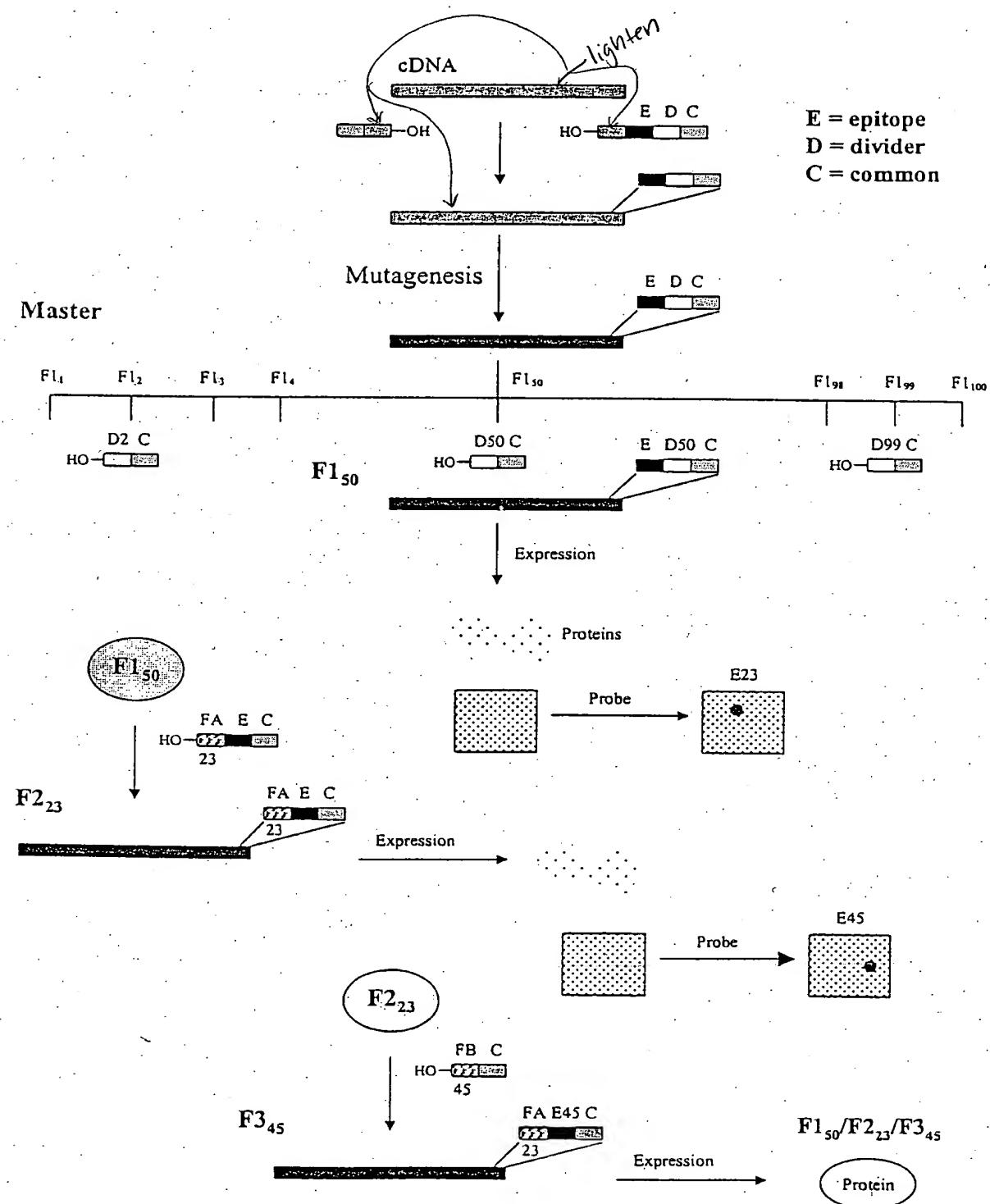
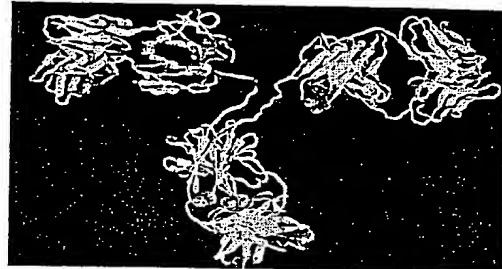
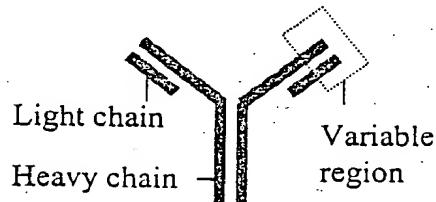


FIGURE 4

Making a recombinant antibody library

Basic antibody structure



Spleen cells or PBLs

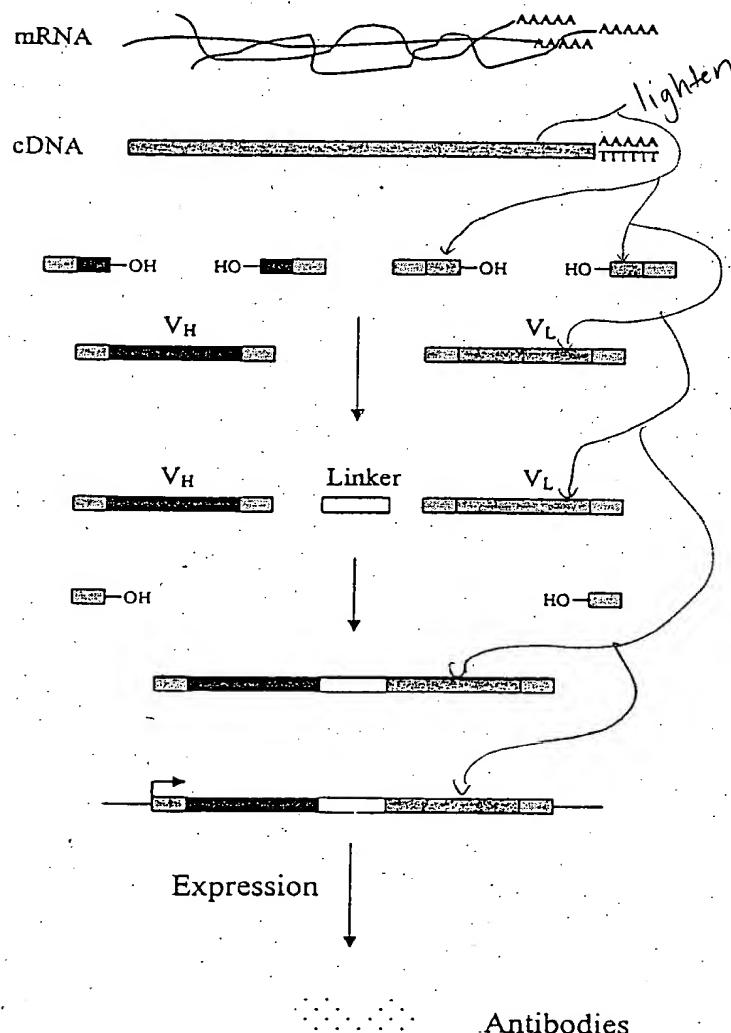


FIGURE 5

Searching a recombinant antibody library

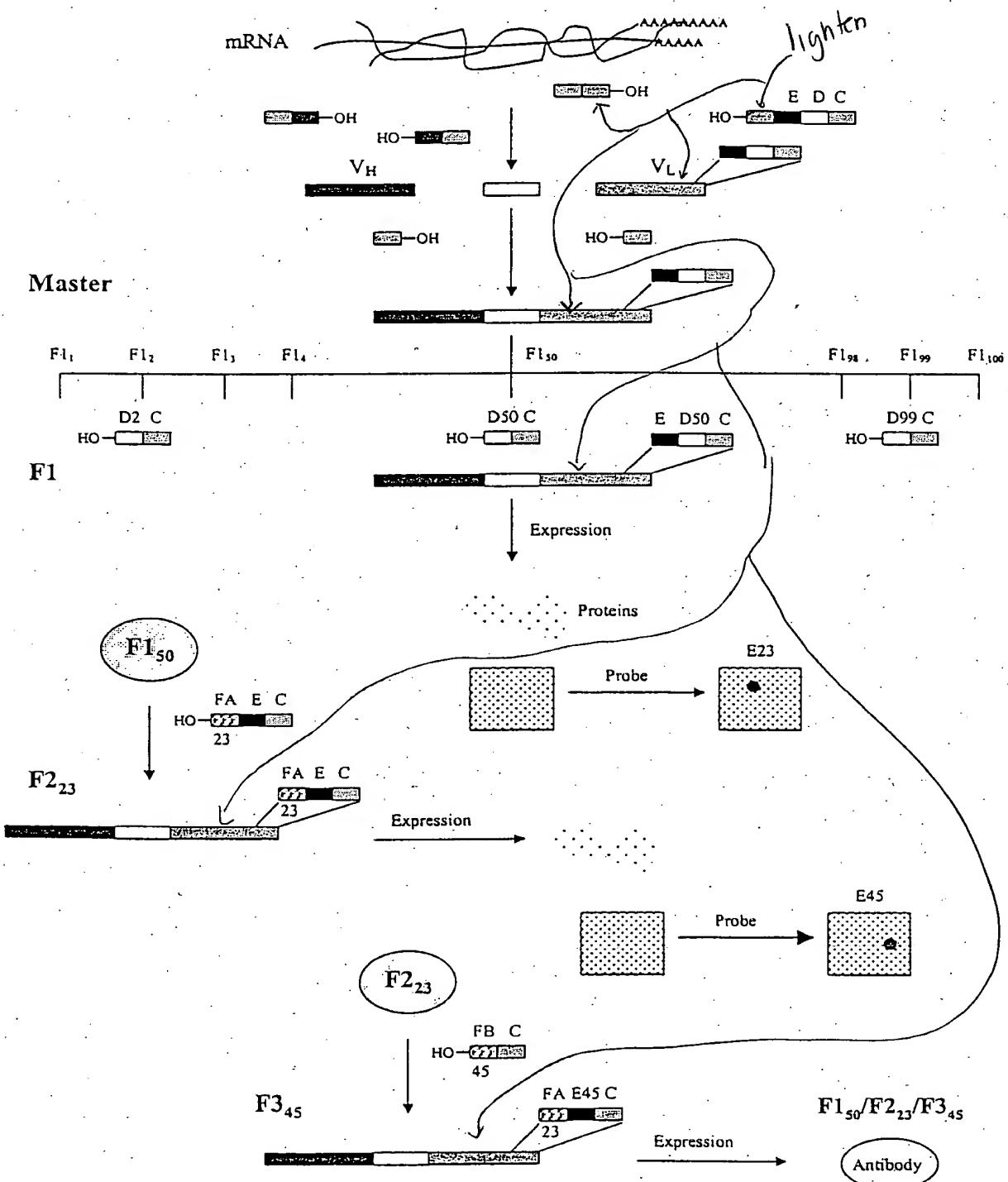
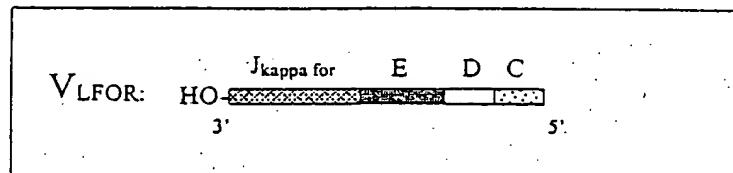


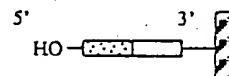
FIGURE 8

Making the VLFOR primers: Solid phase synthesis

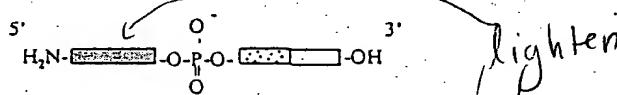


J_{kappa} for Epitope D Common

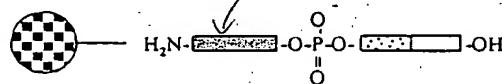
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extend by hybridizing with DNA patch and ligating

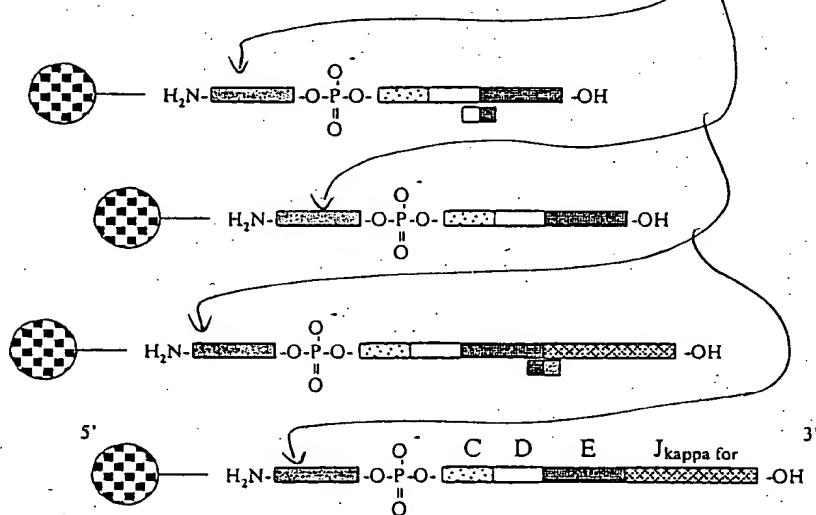
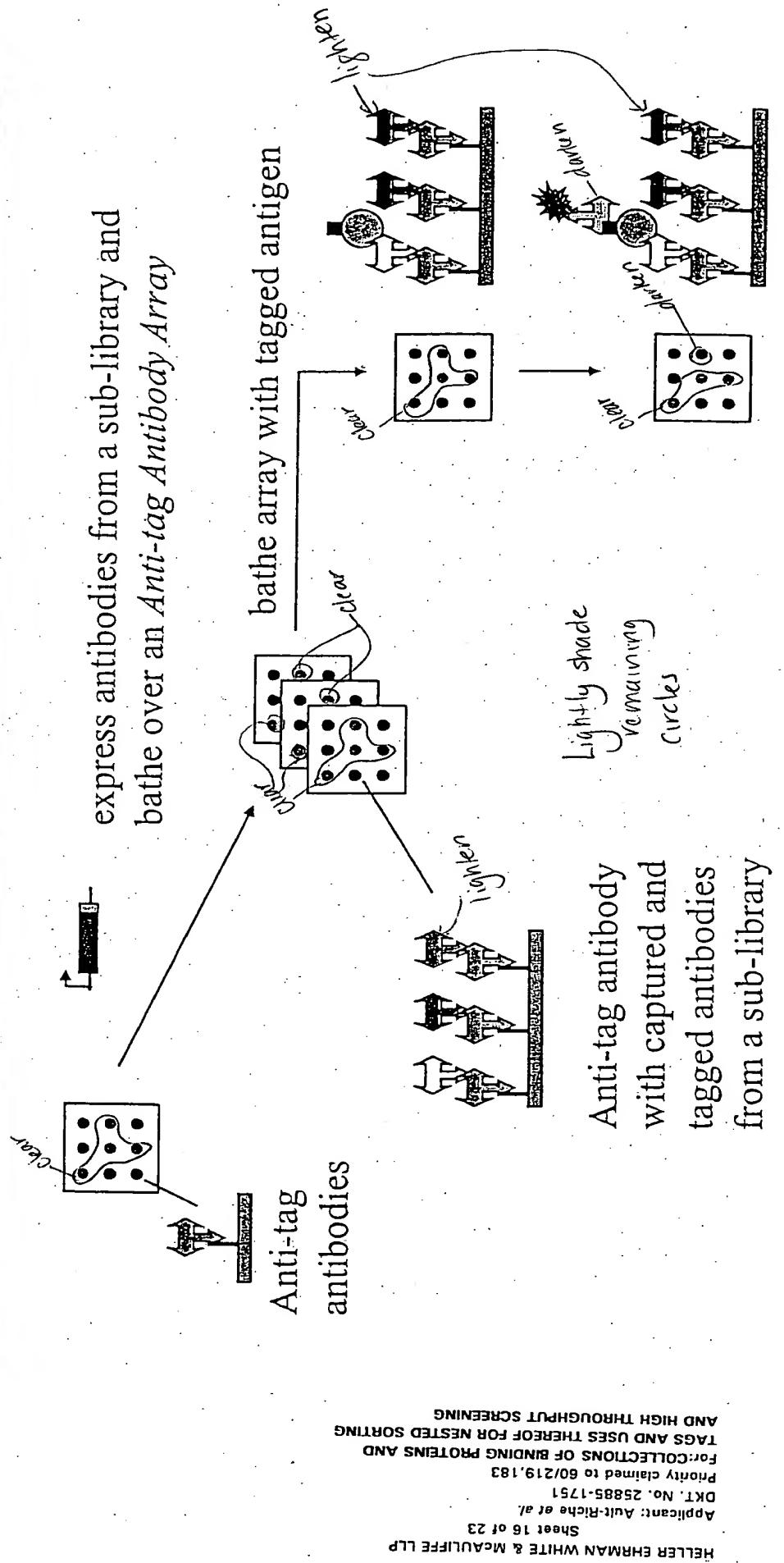


FIGURE 10

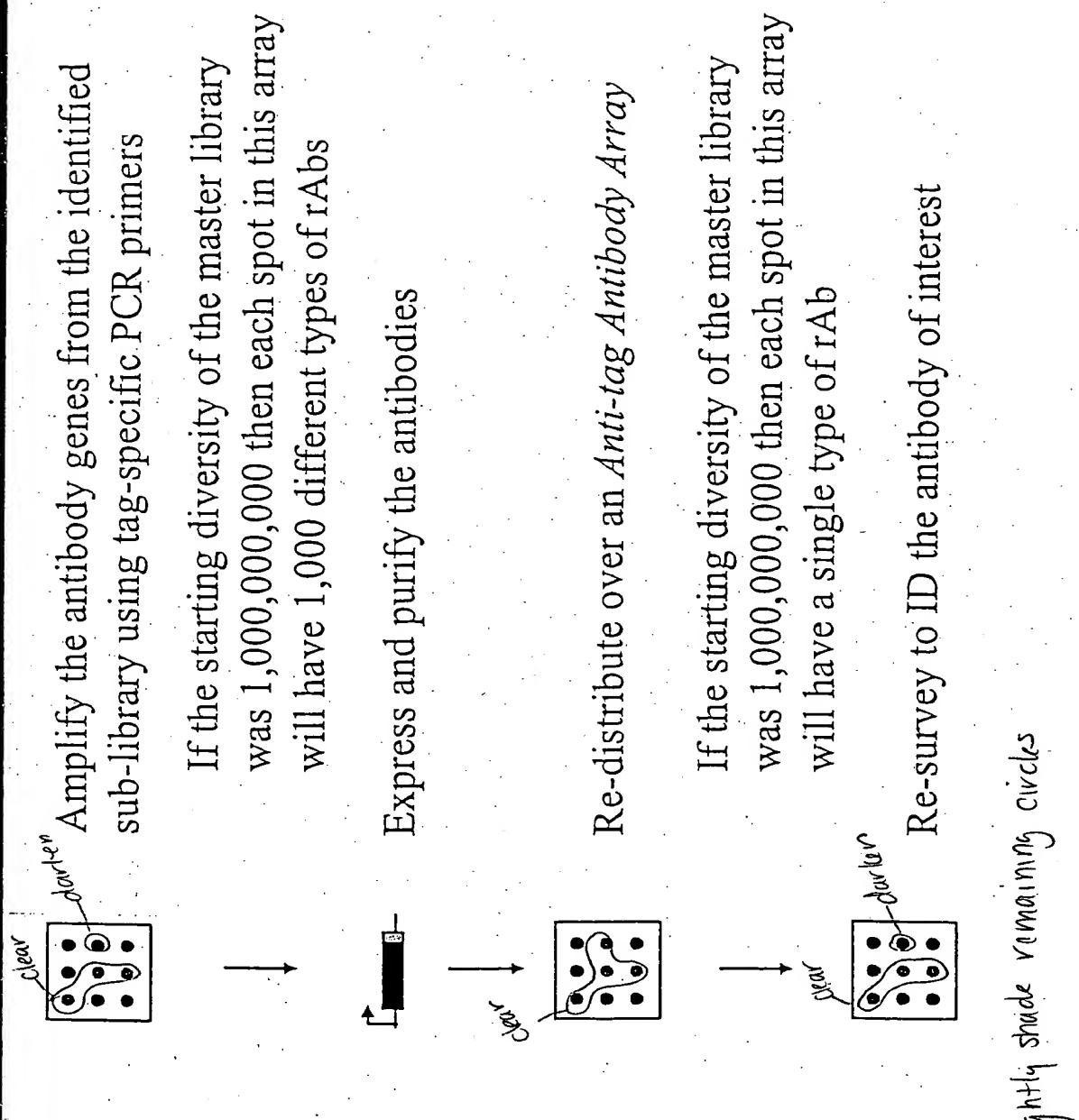
step II



ID spot containing the antigen with a labeled developing Ab

FIGURE 14B

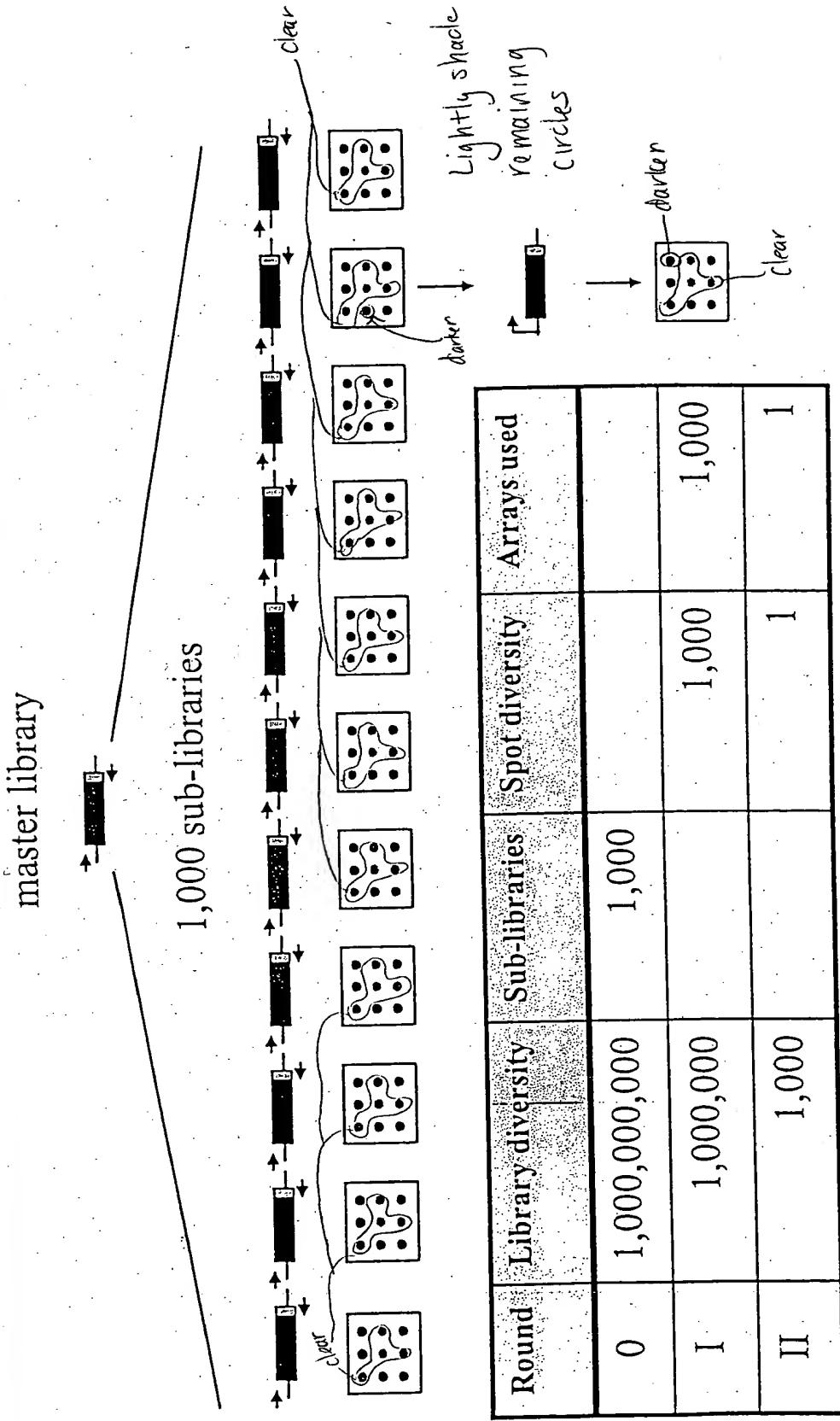
step III



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Sheet 17 of 23
Applicant: Aut-Riche et al.
DKT. No. 25885-1751
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FIGURE 14C

summary



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Sheet 18 of 23
Applicant: Autr-Riche et al.
Dkt. No. 25885-1751
Priority claimed to 60/219,183
For: COLLECTIONS OF BINDING PROTEINS AND
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FIGURE 14D

- Modification searches

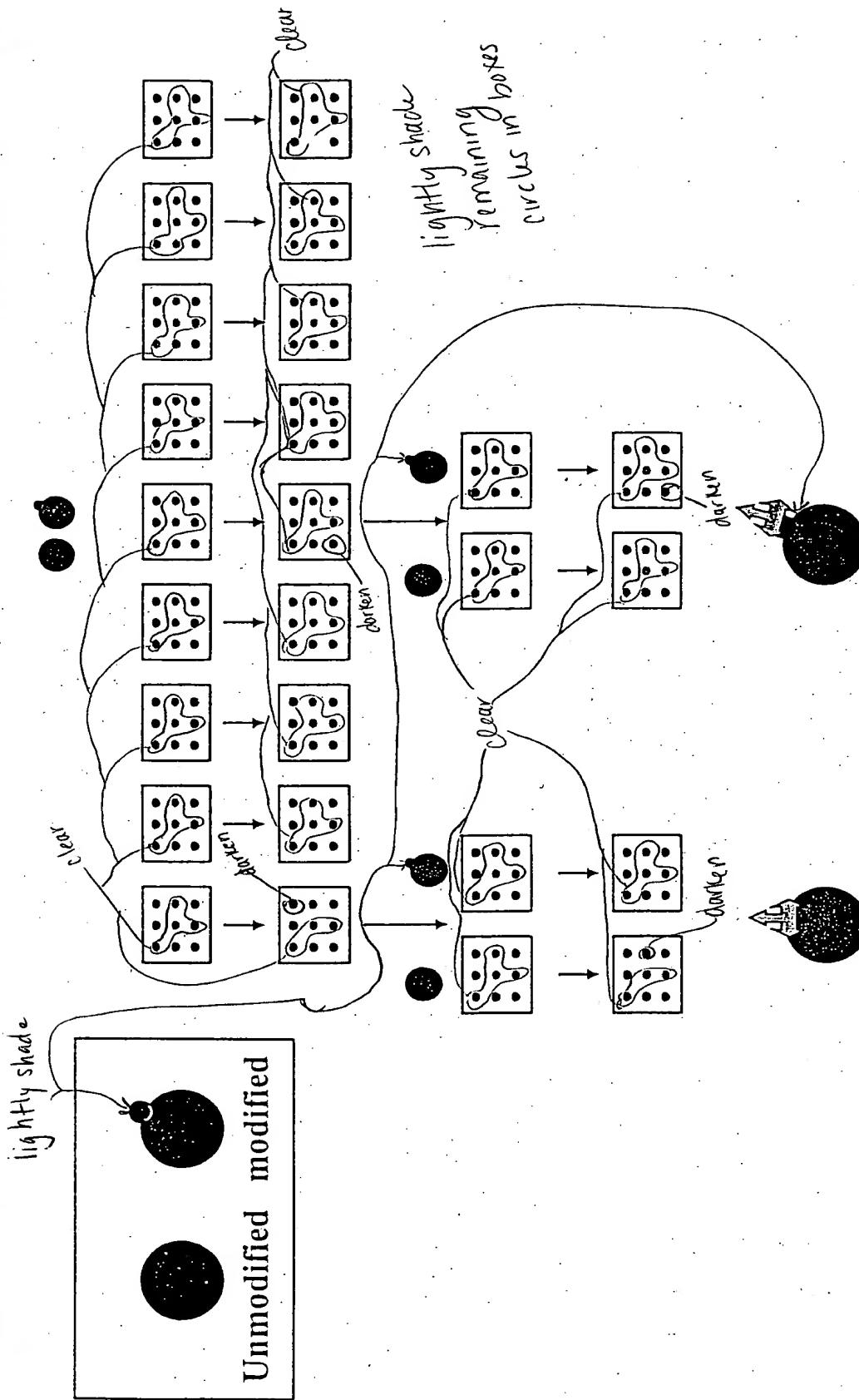


FIGURE 15

HELLER ERMAN WHITE & MACAULIFFE LLP
Sheet 19 of 23
Applicant: Autel-Riche et al.
DKT. No. 25885-1751
Priority claimed to 60/219,183
Protein collections of binding proteins and
tagged uses thereof for nested sorting
tags and high throughput screening
and high throughput screening

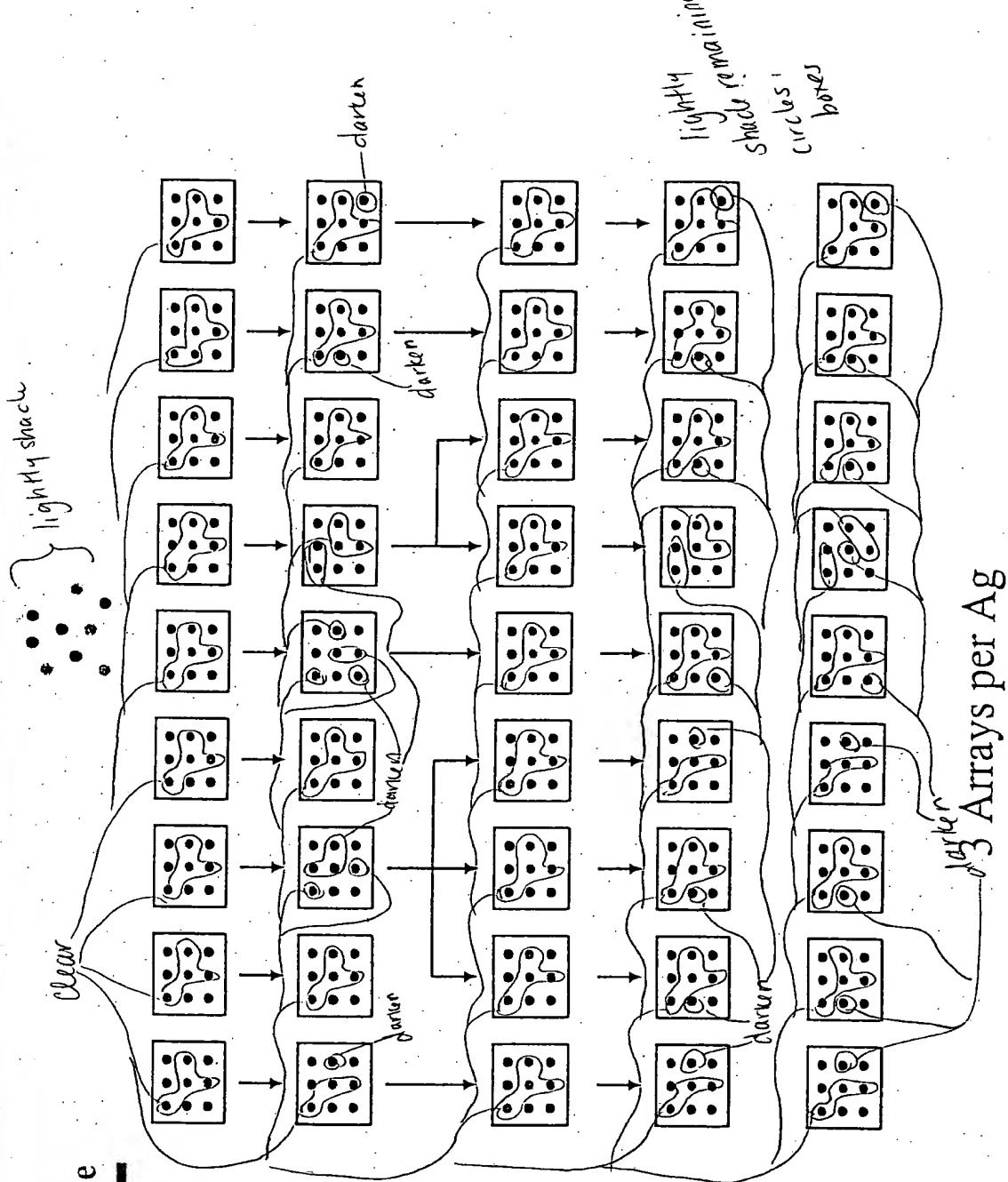
Simultaneous searches

Round Arrays Bait Probe

I 1,000 Abs Ags

II 1,000 Abs Ags

III 1,000 Ags Abs
3,000

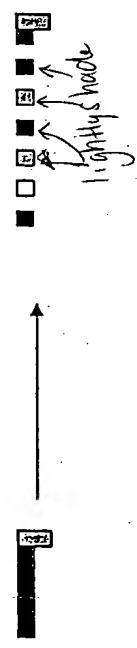


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Sheet 20 of 23
Applicant: Autri-Riche et al.
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For: COLLECTIONS OF BINDING PROTEINS AND
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AND HIGH THROUGHPUT SCREENING
AND HIGH THROUGHPUT SORTING

FIGURE 16

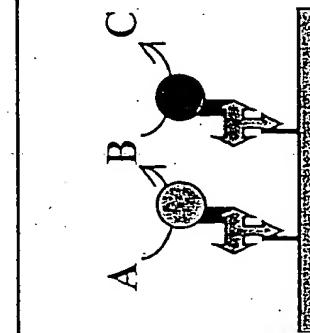
Enzyme engineering

Natural gene(s)
Error-prone PCR
or Gene Shuffling



- tag the gene to be mutated
- mutate genes and create sub-libraries
- distribute mutants over arrays
- probe the arrays with labeled substrates

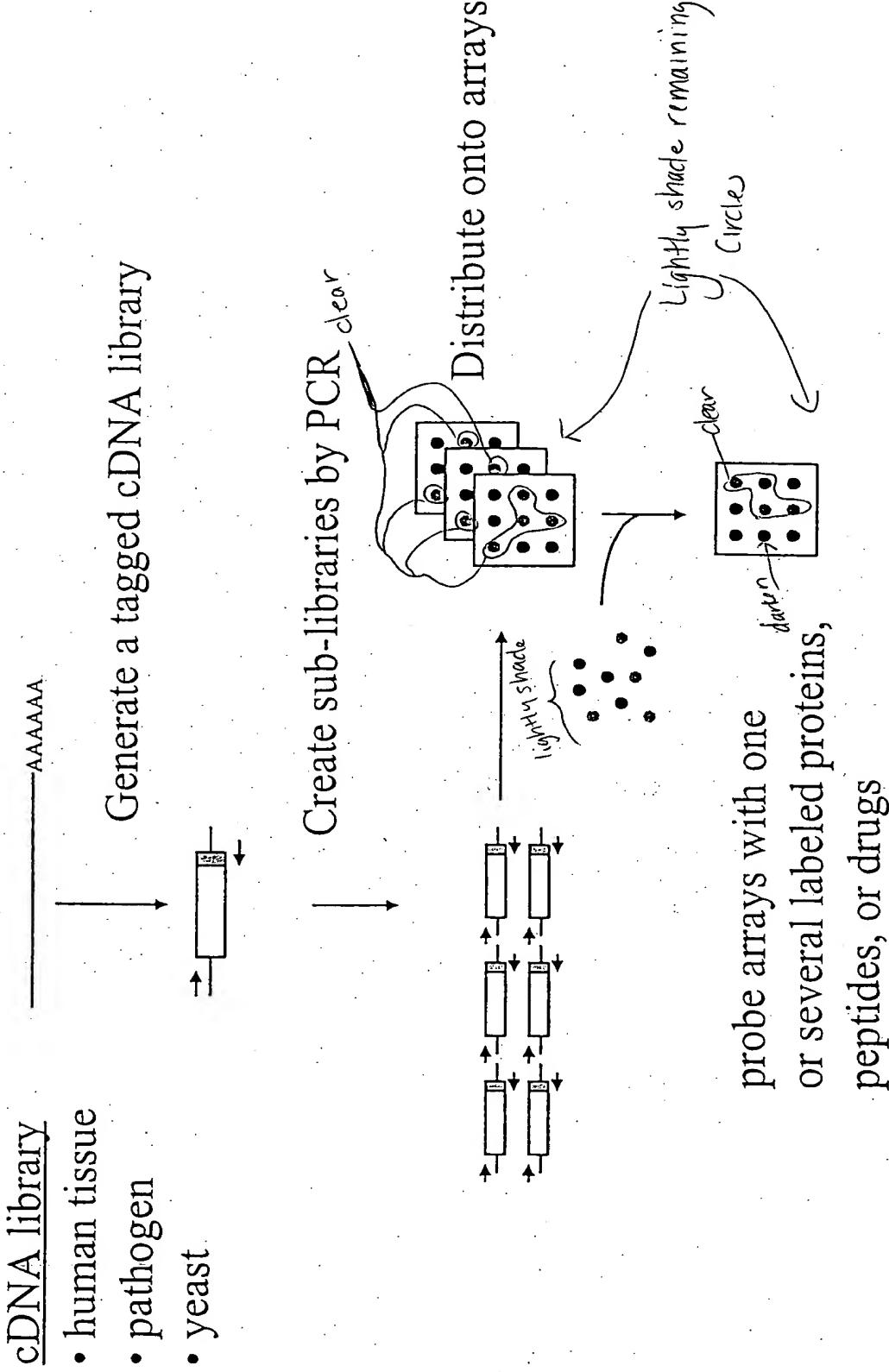
HELLER EHRLICHMAN WHITE & MAGULIFFE LLP
Sheet 21 of 23
Application: Aut-Riche et al.
Dkt. No. 25885-1175
Priority claimed to 60/219,183
For: COLLECTIONS OF BINDING PROTEINS AND
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AND HIGH THROUGHPUT SCREENING



Spots can contain mixtures of enzymes
for detection or pathway engineering

FIGURE 17

Protein interaction mapping



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Sheet 22 of 23
Application: Aut-Riche et al.
Dkt. No. 25885-1751
Priority claimed to 60/219,183
For: COLLECTIONS OF BINDING PROTEINS AND
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AND HIGH THROUGHPUT SCREENING

FIGURE 18

Sorting by pools

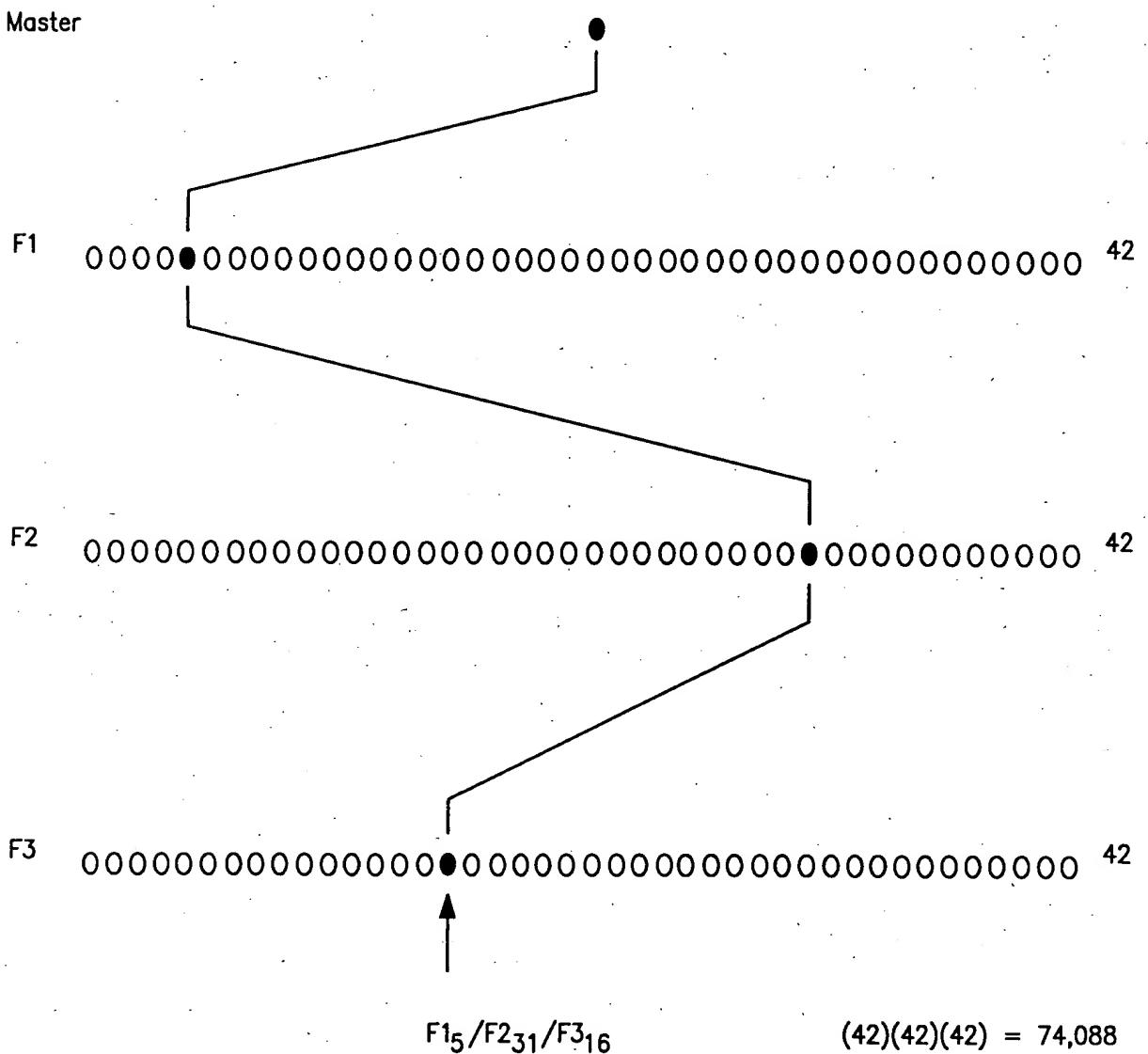


FIG. I

Sorting by pools: Decreasing pool diversities

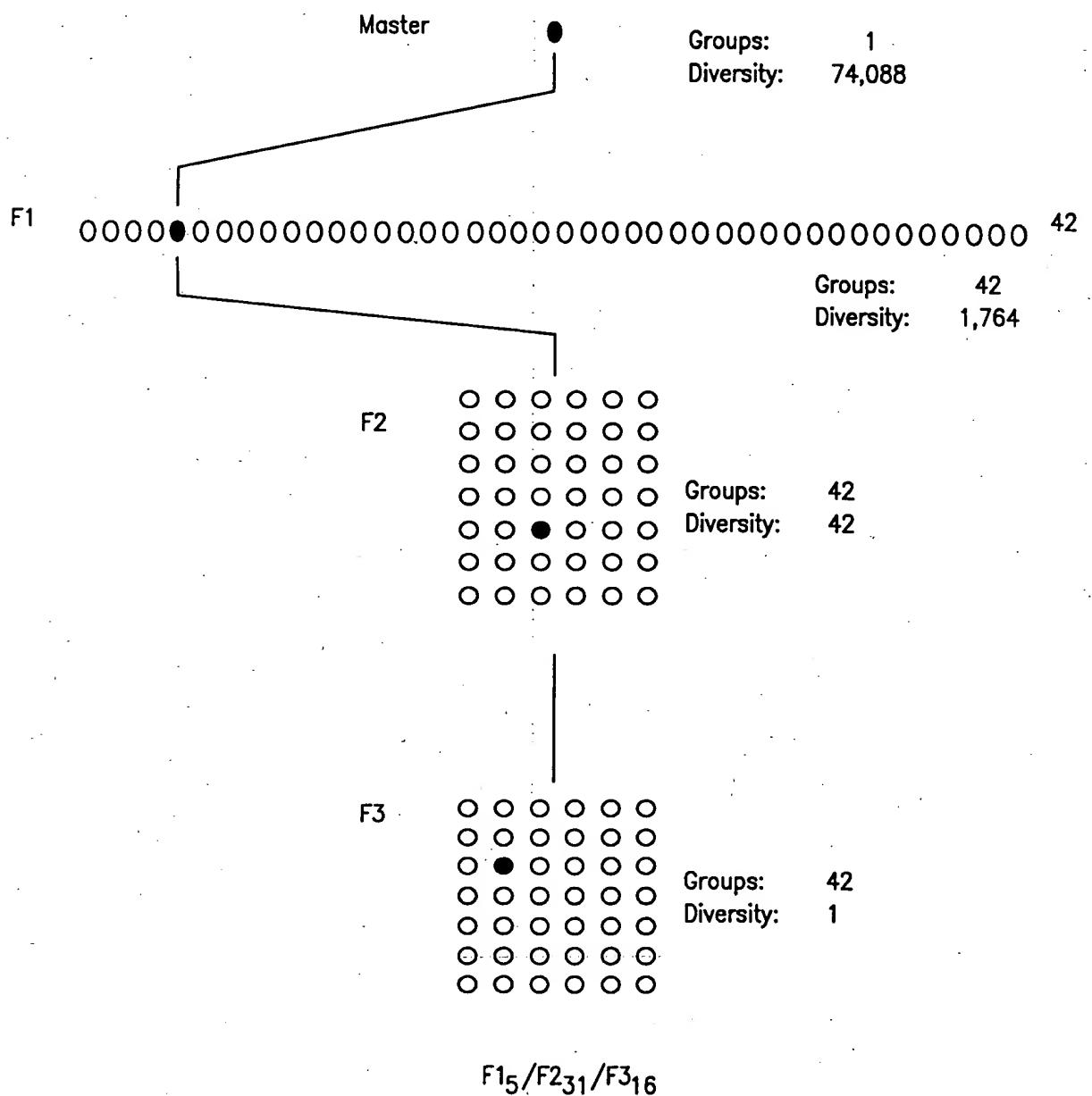


FIG. 2

Sorting by pools: Screening large diversity libraries

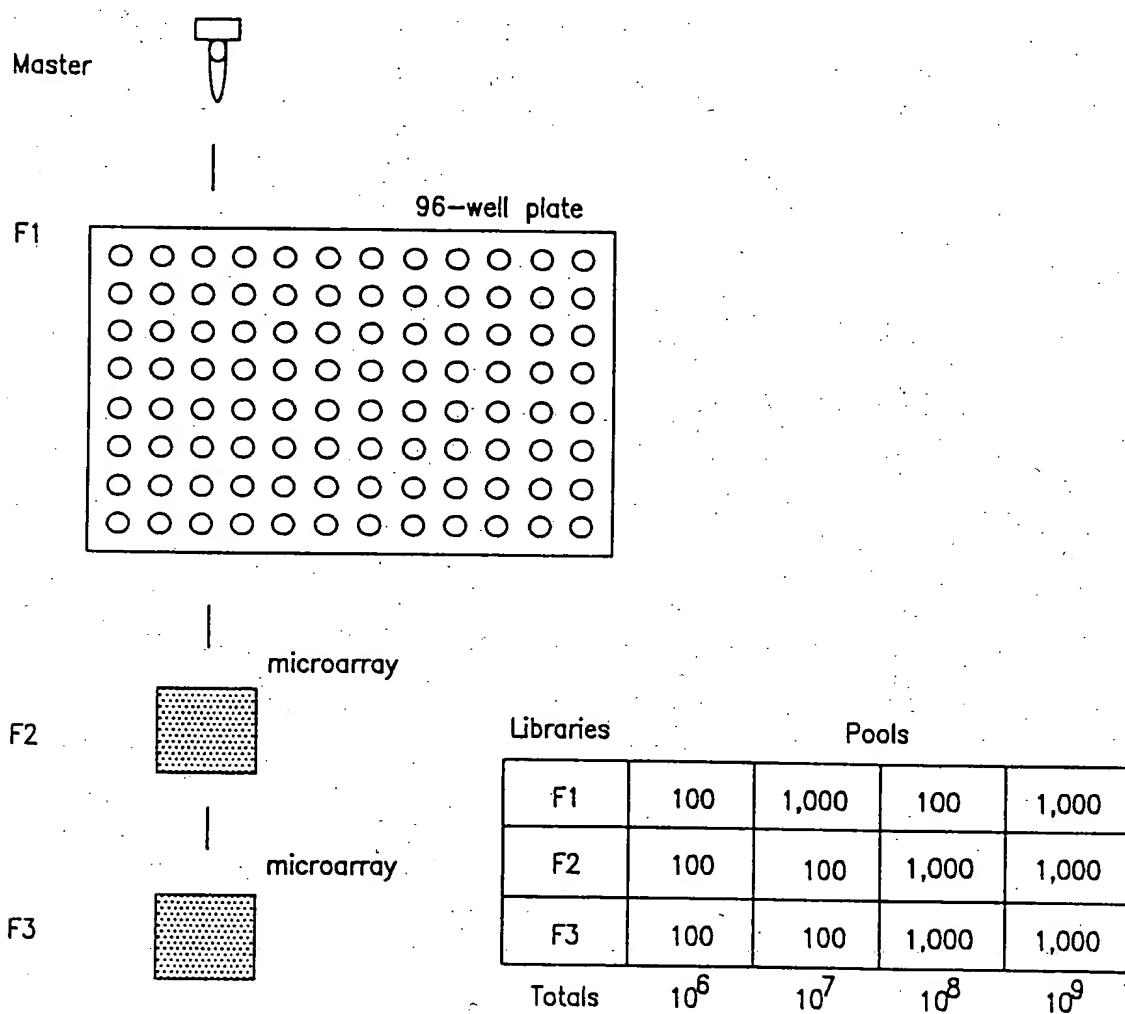


FIG. 3

Searching a mutation library

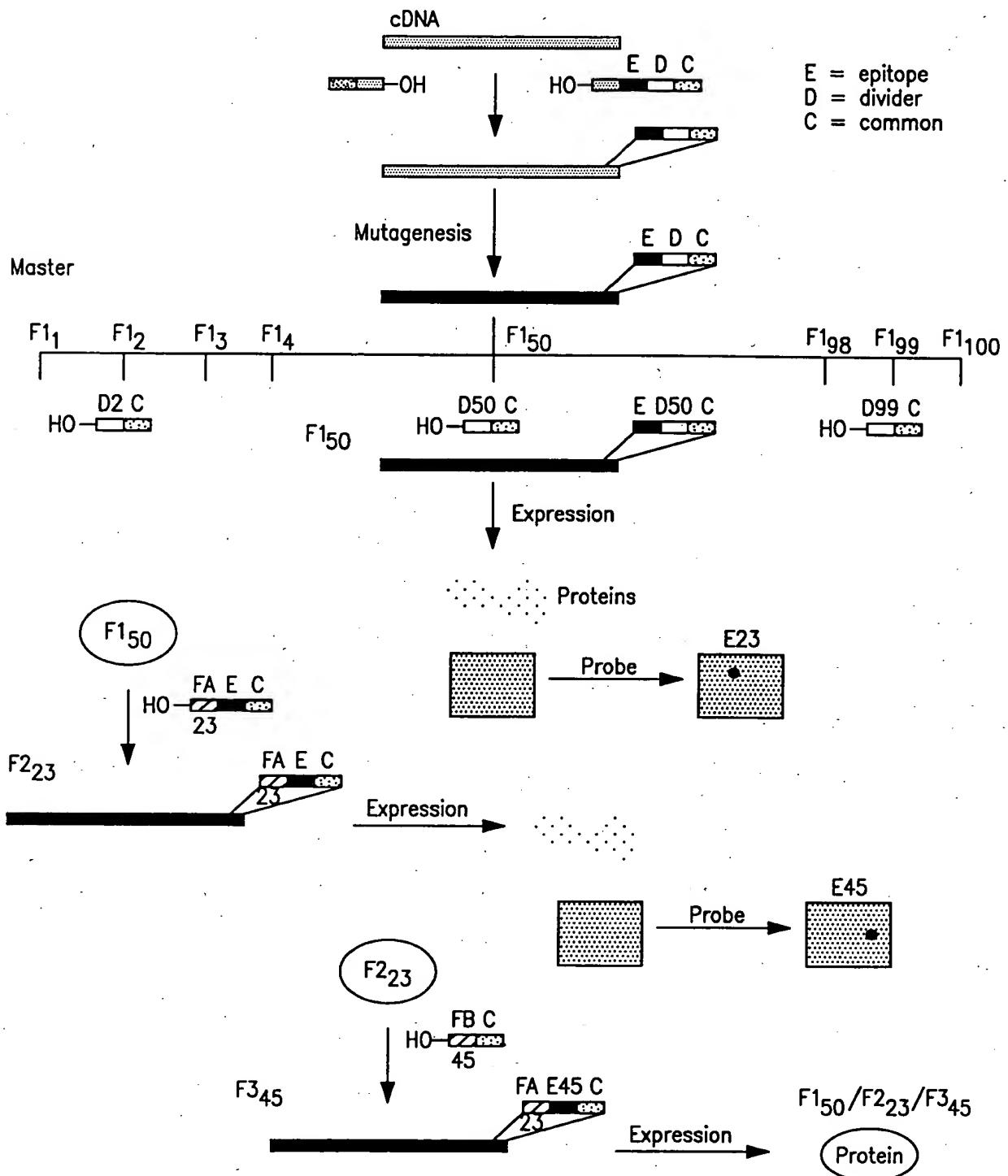
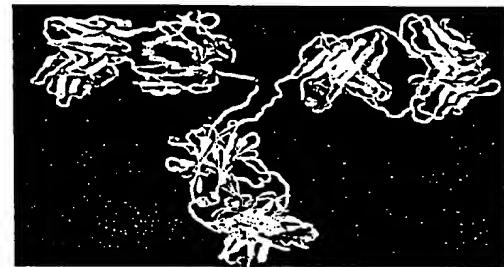
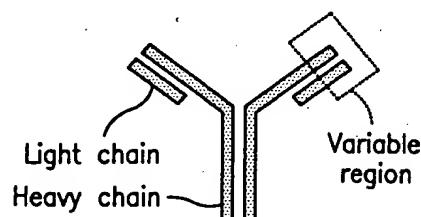


FIG. 4

Making a recombinant antibody library



Spleen cells or PBLs



mRNA

Two wavy lines representing mRNA molecules. Each molecule has a horizontal bar at its right end labeled "AAAAAA" with a vertical line extending downwards, representing a poly-A tail.

cDNA

A single horizontal line representing cDNA, which is complementary DNA. It has a horizontal bar at its right end labeled "AAAAAA" with a vertical line extending downwards, representing a poly-A tail.

 OH HO

V_H

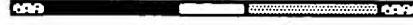
V_L

↓

V_H Linker V_L

↓

HO



↓

Expression

Antibodies

FIG. 5

Searching a recombinant antibody library

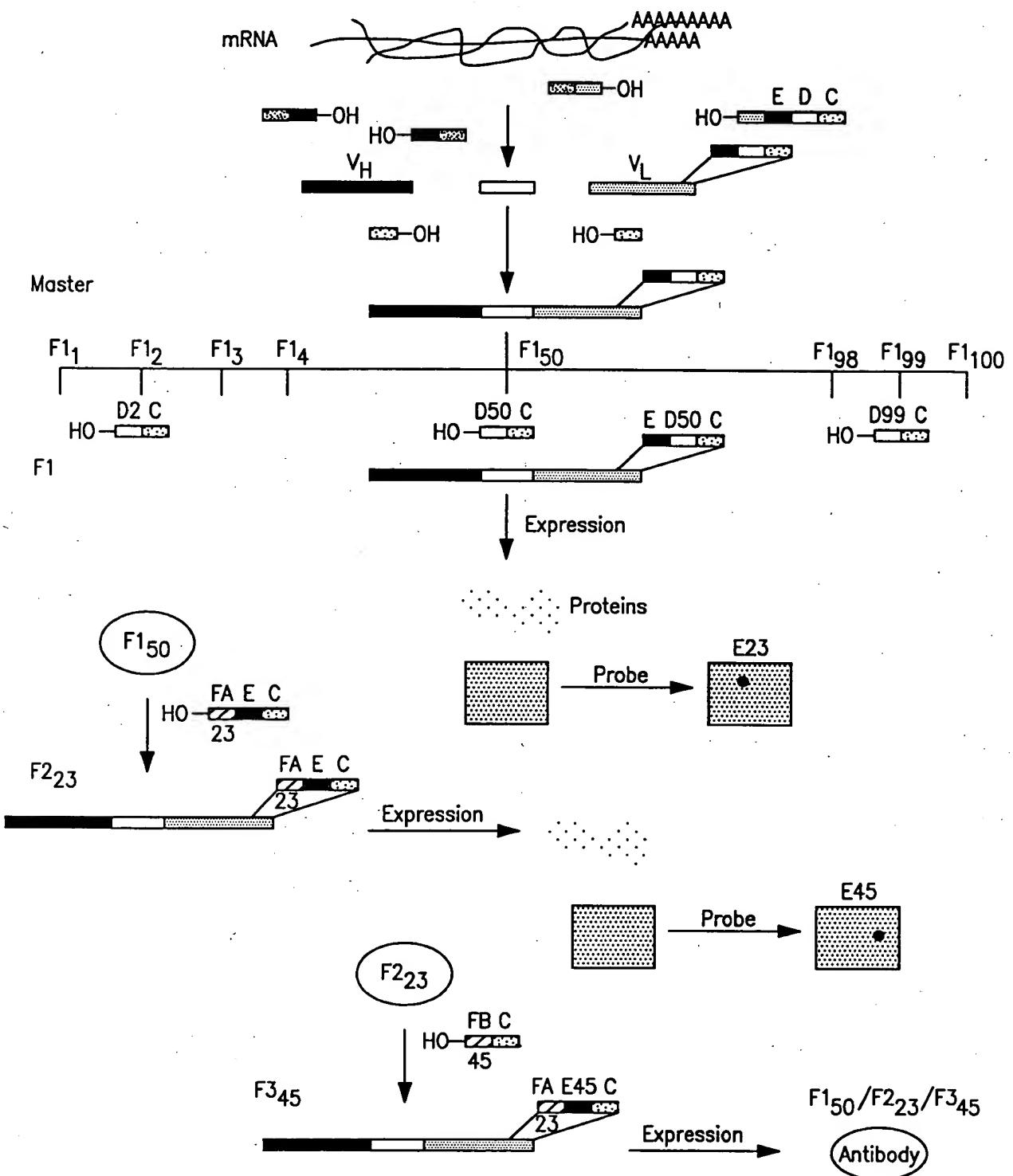
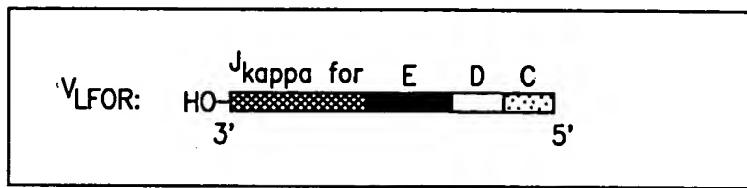


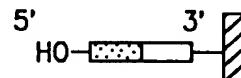
FIG. 8

Making the V_{LFOR} primers: Solid phase synthesis

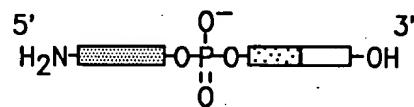


J kappa for Epitope D Common

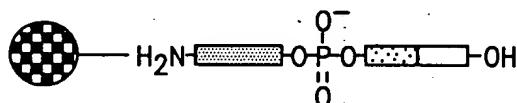
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4. Extended by hybridizing with DNA patch and ligating

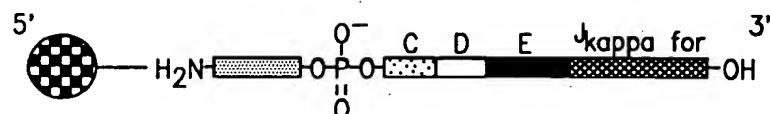
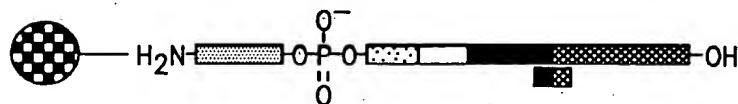
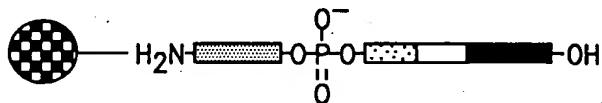
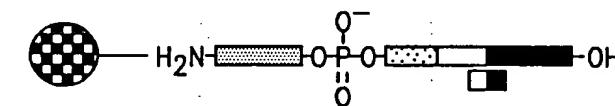


FIG. 10

FIG. I4B

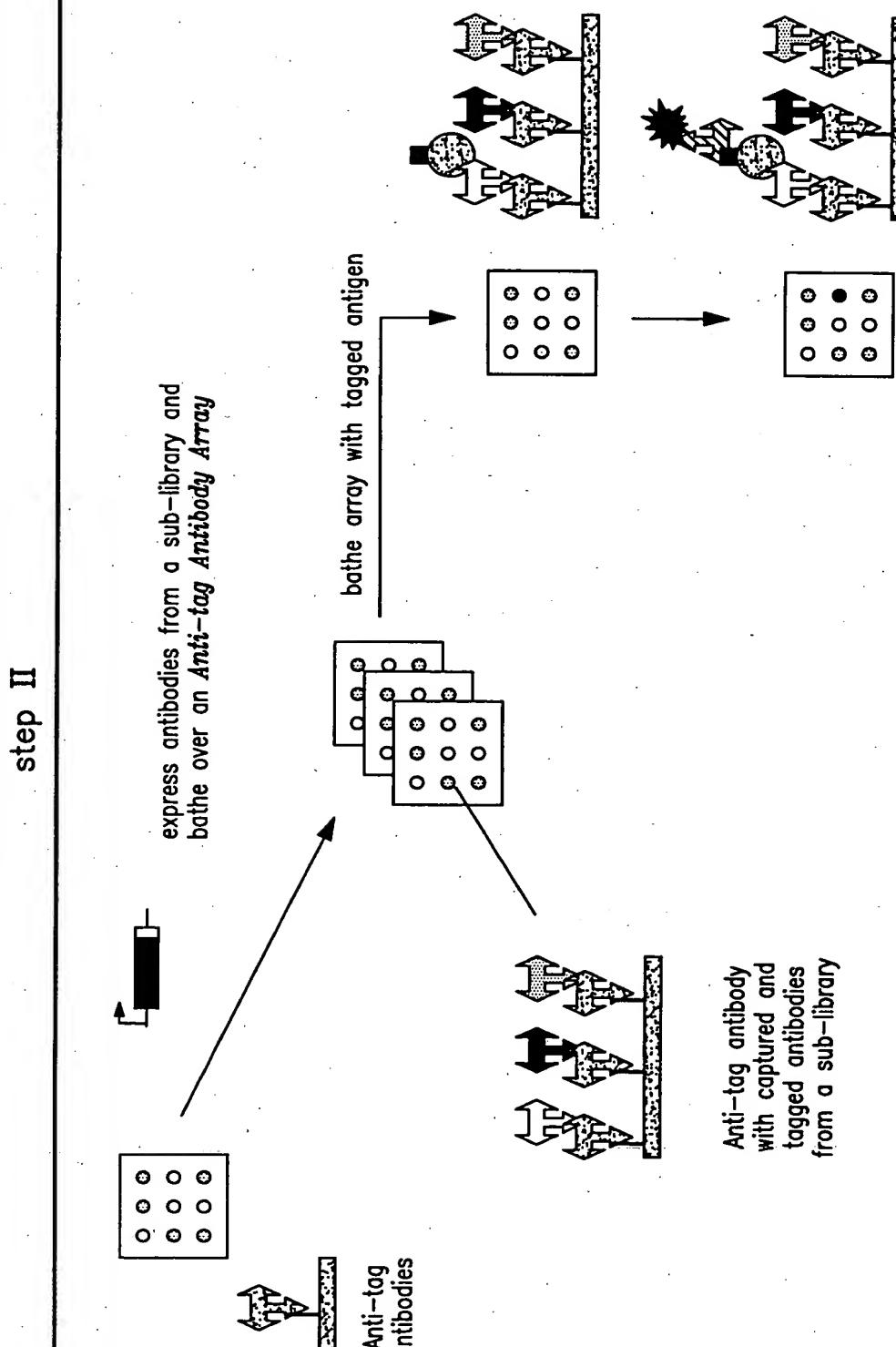


FIG. 14C

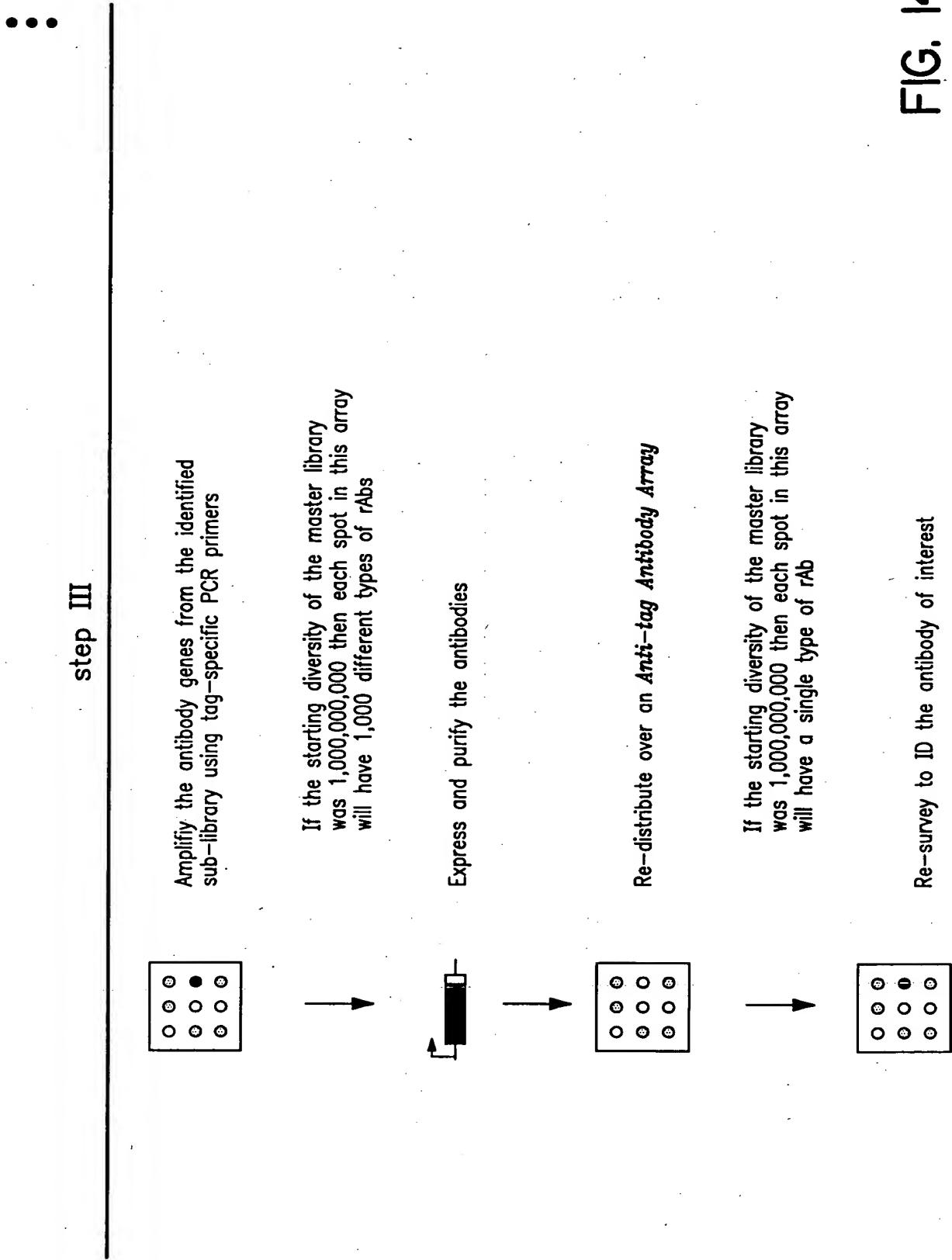


FIG. I4D

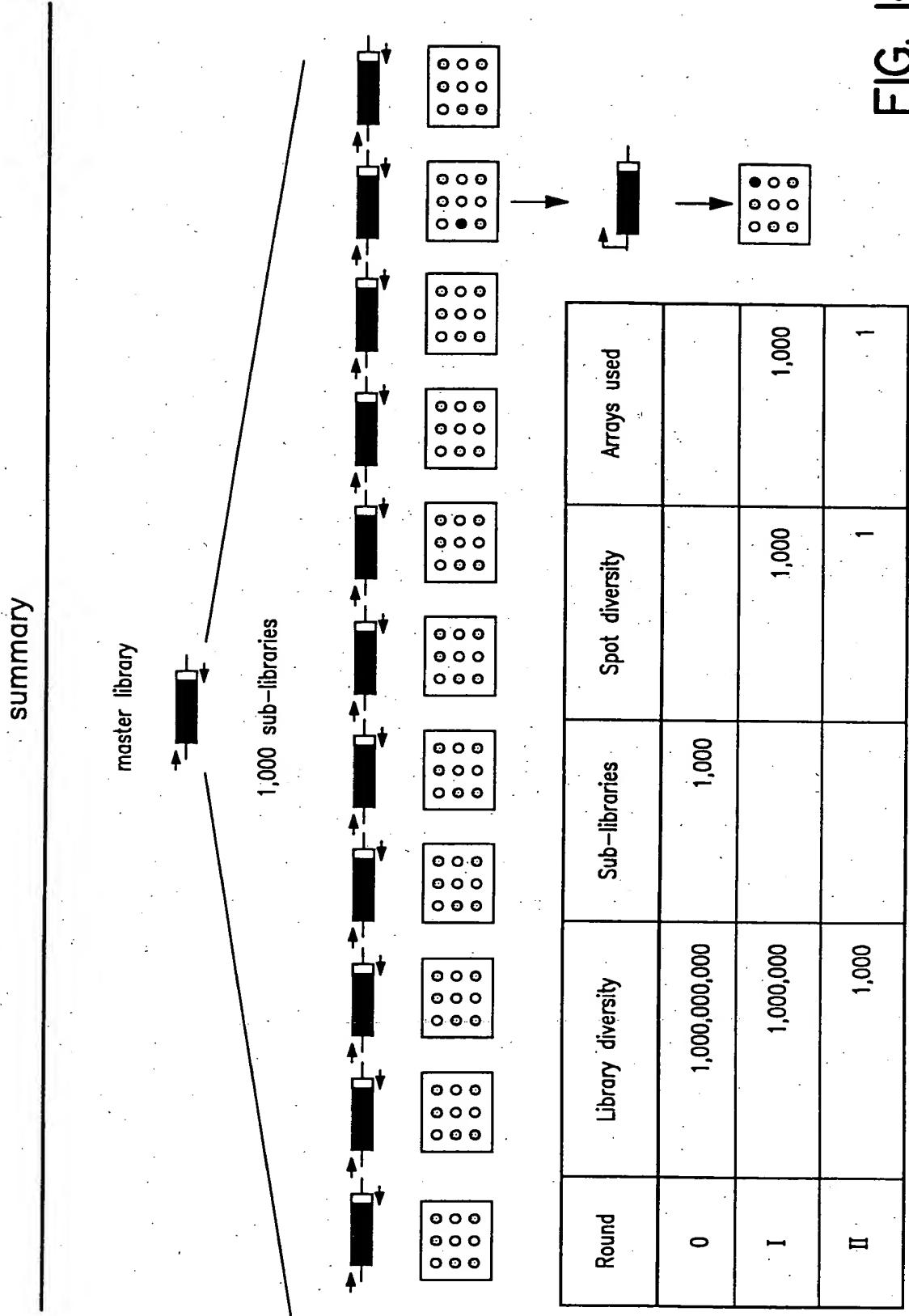


FIG. 15

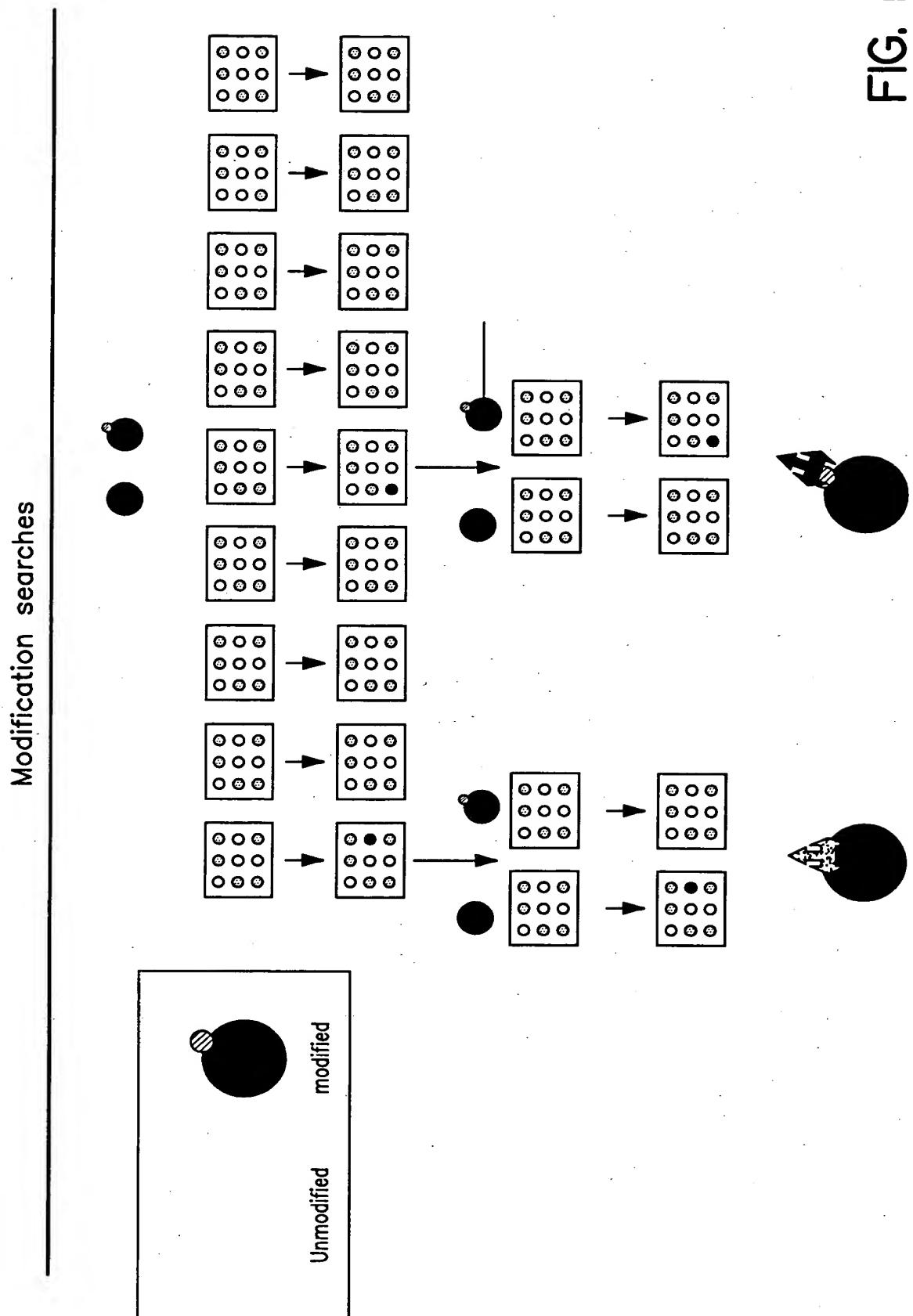
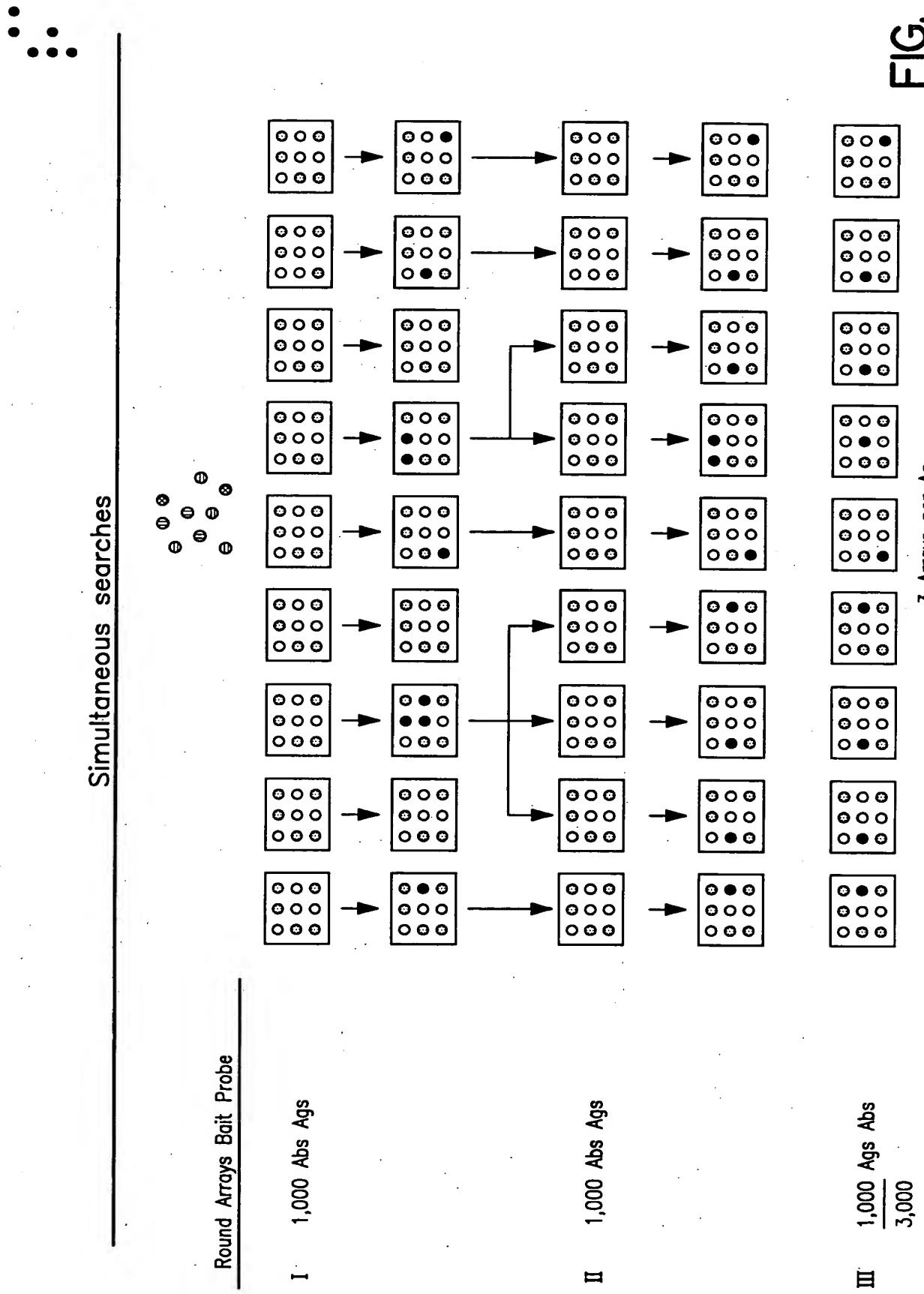
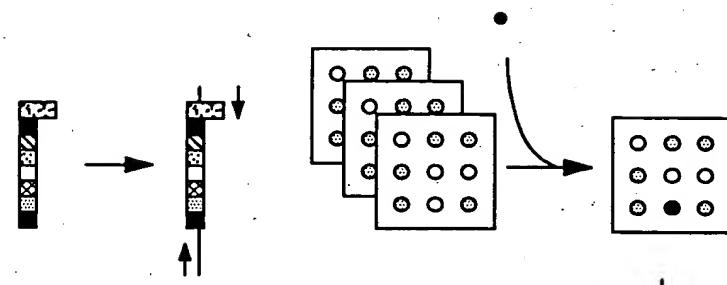
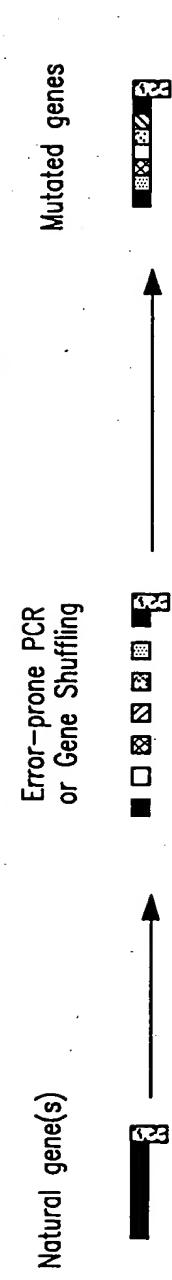


FIG. 16

3 Arrays per Ag



Protein interaction mapping



- tag the genes to be mutated

- mutate genes and create sub-libraries

- distribute mutants over arrays

- probe the arrays with labeled substrates

Spots can contain mixtures of enzymes
for detection or pathway engineering

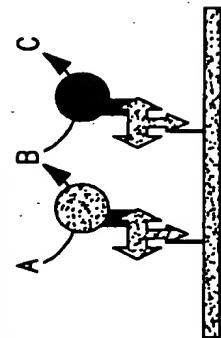


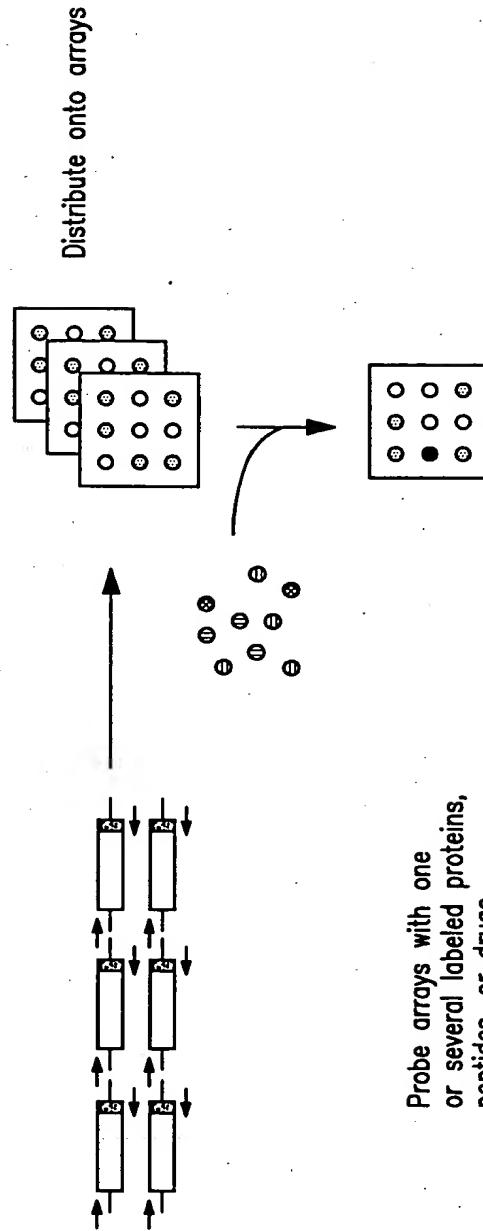
FIG. 17

Protein interaction mapping

cDNA library

- Human tissue
 - pathogen
 - yeast
- Generate a tagged cDNA library
-

Create sub-libraries by PCR



Probe arrays with one
or several labeled proteins,
peptides, or drugs

FIG. 18